BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N

A2

(11) International Publication Number: WO 92/22797

(43) International Publication Date: 23 December 1992 (23.12.92)

(21) International Application Number: PCT/US92/03680

(22) International Filing Date: 5 May 1992 (05.05.92)

(30) Priority data:
715,407 13 June 1991 (13.06.91) US
715,119 14 June 1991 (14.06.91) US

(71) Applicant: PACIFIC BIOTECH, INC. [US/US]; 9050 Camino Santa Fe, San Diego, CA 92121 (US).

(72) Inventors: TZENG, Sinfu ; 450 Crestlake Drive, San Francisco, CA 94132 (US). WANG, Dou-Mei ; 2216 Silver Park Peak Place, Encinitas, CA 92024 (US).

(74) Agents: ALTMAN, Daniel, E. et al.; Knobbe, Martens, Olson and Bear, 620 Newport Center Drive, Suite 1600, Newport Beach, CA 92660 (US).

(81) Designated States: AT (European patent), AU, BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, KR, LU (European patent), MC (European patent), NL (European patent), NO, SE (European patent).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: ASSAY FOR THE DETECTION OF SPECIFIC LIGANDS

(57) Abstract

The invention disclosed herein relates to the field of one-step assays and particle agglutination tests. In one embodiment of the present invention, there is disclosed an analytical device for the detection or determination of an analyte antibody in a bodily fluid comprising a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, the layer including a sample application zone, a conjugate zone containing antigen bound to mobile particles, and a detection zone containing immobilized antigen, wherein the antigen is the same in both the conjugate and detection zones and is an antigen that binds with the analyte antibody, the liquid sample is capable of moving from the sample application zone through the conjugate zone and on to the detection zone, and if the analyte antibody is present in the sample it is detected in the detection zone. The present invention also discloses methods of detecting various analytes, particularly analyte antibodies, including specific immunoglobulins. The invention further provides improved methods of preparing and using one-step assays, as well as improved methods of preparing coated particles for use in diagnostic assays.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FI	Finland	ML	Mali
AU	Australia	FR	France	MN	Mongolia
BB	Barbados	GA	Gabon	MR	Mauritania
BE	Belgium	GB	United Kingdom	MW	Malawi
BF	Burkina Faso	GN	Guinea	NL	Netherlands
BG	Bulgaria	GR	Greece	NO	Norway
BJ	Benin	HU	Hungary	PL	Poland
BR	Brazil	IE	Ireland	RO	Romania
CA	Canada	IT	Italy	RU	Russian Federation
CF	Central African Republic	JP	Japan	SD	Sudan
CG	Congo	KP	Democratic People's Republic of Korea	SE	Sweden
CH	Switzerland			SN	Senegal
CI	Côte d'Ivoire	KR	Republic of Korea	SU	Soviet Union
CM	Cameroon	LI	Liechtenstein	TD	Chad
CS	Czechoslovakia	LK	Sri Lanka	TG	Togo
DE	Germany	LU	Luxembourg	US	United States of America
DK	Denmark	MC	Monaco		
ES	Spain	MG	Madagascar		

ASSAY F R THE DETECTION OF SPECIFIC LIGANDS

RELATION TO RELATED APPLICATION

This application is a continuation-in-part of the U.S. Patent Application, serial no. unknown, filed June 13, 1991, naming Tzeng, et al., as inventors. Priority of subject matter in this application common with subject matter in that application is hereby claimed.

BACKGROUND OF THE INVENTION

Recent technological advances have made it possible to tailor assays for a wide variety of analytes, especially those molecules exhibiting antigenic characteristics, such as polypeptides, nucleotides, whole cells, and cellular fragments, to name but a few. In general, most assays currently in use tend to use antibodies to "capture" antigenic materials in a liquid-phase or a solid-phase format.

Nevertheless, assays with an improved sensitivity are needed, as many conditions and diseases do not lend themselves to early diagnosis via antigenic detection. In many instances, it would be preferable to be able to detect an increase in a specific population of cells or molecules in an organism, which population is produced in response to an "invasion" of the organism by a specific antigenic material.

For example, in the case of certain viral diseases, by the time a sufficiently detectable titer of viral particles is present in an organism's blood, the time for effective therapy may well have passed. In addition, diseases that trigger highly specific, but virtually undetectable, responses by an organism's immune system, do not lend themselves to accurate or easy detection via currently-available assays. Moreover, agents which provoke a response by a specific population or subpopulation of immunoglobulins are often not detectable until the resulting disease is full-blown, thus limiting diagnostic, as well as therapeutic, options. For example, an assay claiming to facilitate the differential detection of

various immunoglobulin species from each other is described in U.S. Patent No. 4,292,403 to Duermeyer. Specifically, this assay purports to detect an antigen-specific immunoglobulin of a particular immunoglobulin class, which classes include IgM, IgG, IgA, IgD, or IgE, by using anti-antibodies against the specific immunoglobulin class. Like many other assays disclosed in the art which use anti-antibodies (e.g., U.S. Patent No. 4,818,688 to Adamich, et al., No. 4,828,981 to Maggio, et al., and No. 4,962,023 to Todd, et al.), the assay described by Duermeyer is complex and involves a multiplicity of specific reagents. Furthermore, none of these assays can easily distinguish an antigen-specific subpopulation of an immunoglobulin class from other, non-antigen-specific members of its class. That is, prior to the advent of the present invention, it has not been a simple matter to detect subpopulations of specific IgG or IgM molecules, for example, which are precisely sensitized to a single antigenic substance.

An illustrative example of the need for assays with increased sensitivity is provided by consideration of available assays for the detection of antibodies, and in particular, for the detection of immunoglobulins directed against specific antigens. Lyme Disease provides such an illustrative example.

Lyme Borreliosis was first identified in Lyme, Connecticut in 1975. It has now been reported across several continents, including North America, Europe, Russia, Asia, Africa and Australia. The disease is caused by the tick-transmitted spirochete *Borrelia burgdorferi*. This infection can produce a wide spectrum of clinical symptoms which can be confused with other entities. Therefore, precise diagnosis is critical.

Lyme disease is the most commonly reported tick-borne illness in the United States. The disease is most prevalent

in the northeast, upper midwest, and west coast states. The seasonal onset of the disease is synchronous with the nymphal stage of the ticks. Thus, the disease is more common in summer and early fall.

Antibody titers specific to *Borrelia burgdorferi* are typically negative during early illness. Patients with only erythema migrans also rarely have elevated antibody titers. The specific IgM levels begin to rise two weeks after the onset of the disease and peak at three to six weeks. The specific IgG levels tend to lag behind the specific IgM titers by approximately two weeks, but are often positive during the latter part of the clinical stage of erythema migrans and usually remain positive during the second and third clinical stages with manifestations of carditis, neurological disease or arthritis.

Generally, the "natural history" or progression of Lyme disease may be divided into three clinical stages. The first stage is characterized by the development of an expanding annular red rash-like skin lesion, erythema migrans, which occurs at the site of the tick bite and typically lasts two to four weeks. The erythema migrans may be followed by cardiac, joint and neurological abnormalities in the second stage, which occurs one to four months after the disease onset. The last stage is characterized by arthritis involving a few large joints which may begin as early as three months after onset. This stage can last several years or may become chronic. Early diagnosis permits prompt treatment with appropriate antibiotics that can halt the progression of the disease.

Since the spirochete is often difficult to culture from affected skin or body fluids of patients, serological detection of antibodies is considered the best available diagnostic means for Lyme disease. The specific IgM against *Borrelia burgdorferi* is often not detectable during the first two weeks, but it usually peaks three to six weeks after the initial infection, and then persists or declines. The

response of the specific IgG to the spirochete is frequently not detectable for four to six weeks, but peaks in the arthritic stage and often remains elevated for years.

Therefore, in response to an express need for an assay procedure with diverse applicability, which is capable of detecting specific ligands, including various polypeptides, polynucleotides, and immunoglobulins, with great sensitivity, Applicants disclose the present invention. In addition, the presently-described assays avoid the agglomeration problems of other assays, which promotes the goals of improved accuracy and greater resolution. The present invention, which is elegant in its simplicity, is hereby disclosed by Applicants, including its equivalents thereof.

SUMMARY OF THE INVENTION

In one embodiment of the present invention, Applicants disclose an analytical device for the detection or determination of an analyte antibody in a bodily fluid comprising a layer of a plurality of substantially planar zones adjacent one another and in absorbent contact with one another, the layer including a sample application zone, a conjugate zone containing antigen bound to mobile particles, and a detection zone containing immobilized antigen, wherein the antigen is the same in both the conjugate and detection zones and is an antigen that binds with the analyte antibody, the liquid sample is capable of moving from the sample application zone through the conjugate zone and on to the detection zone, and if the analyte antibody is present in the sample it is detected in the detection zone.

In another embodiment, the mobile particles are colored plastic particles or a metal sol. Another variation discloses a device wherein the antigen will bind to antibodies which in turn bind to epitopes of the *Borrelia burgdorferi* microorganism. In another variation, the mobile particles are colored polystyrene microparticles. In yet another embodiment, the layer is made from nitrocellulose.

The present invention also discloses a method employing the disclosed devices, which compris s adding sample suspected of containing the analyte antibody to the sample application zone and waiting for sufficient time for the sample to traverse the layer through the detection zone, and reading the results in the detection zone. In one variation, the mobile particles are colored plastic particles or a metal sol. In another variation, the antigen used will bind to antibodies which in turn bind to epitopes of the *Borrelia burgdorferi* microorganism. Yet another aspect discloses that the mobile particles are colored polystyrene microparticles. In another embodiment, the layer is made from nitrocellulose.

The present invention further discloses a process for the determination of the presence or concentration of an analyte antibody in a sample fluid which comprises contacting a sample of the fluid with a first antigen for the analyte antibody, wherein the first antigen is labelled, in order to form a soluble complex between the first antigen and the analyte antibody; contacting the soluble complex with a second antigen, wherein the second antigen is bound to a solid phase insoluble in the fluid, in order to form an insoluble complex of the first antigen, the analyte antibody, and the second antigen; separating the solid phase from the fluid sample and the unreacted, first antigen; measuring either the first, labelled antigen associated with the solid phase of the unreacted amount of the first, labelled antigen; relating the amount of first, labelled antigen measured for a control sample prepared in accordance with the first four steps, the control sample being free of the analyte antibody, to determine the presence of the analyte antibody in the fluid sample, or relating the amount of first, labelled antigen measured for the fluid sample with the amount of labelled antigen measured for samples containing known amounts of analyte antibody prepared in accordance with the first four steps in order to determine the concentration of the analyte antibody in the fluid sample; wherein both the first and

second antigen are the same before they are labelled or attached to the solid phase; respectively. Those skilled in the art will realize that, in the case where the label is a visible particle, such as a gold sol or colored or colorable microparticle, the complex formed by the labelled antigen and the analyte antibody may be insoluble.

In yet another embodiment, the label is an enzyme or a radioisotope. In another alternative embodiment, the antigen reacts with antibodies that in turn react with epitopes of *Borrelia burgdorferi*. Other variations disclose that the label is alkaline phosphatase and the solid phase is a bead, the inner walls of a test tube or the wells of a microtitre plate; alternatively, the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

The present invention further discloses an assay kit comprising a first antigen bound to a solid phase insoluble in the fluid to be tested and a reagent containing a second antigen bound to a label, wherein the solid phase and the reagent are present in sufficient amount to perform at least one assay for analyte antibody in the fluid, and wherein the first and second antigen are the same before they are bound to the solid phase or labelled, respectively. In another variation, the label is an enzyme or a radioisotope. In yet another embodiment, the antigen is an antigen that binds to antibodies which in turn bind to epitopes of *Borrelia burgdorferi*. Another variation discloses that the solid phase is a bead, the inner walls of a test tube or a non-chromatographic apparatus, and the label is alkaline phosphatase. In another embodiment, the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

In another embodiment, an assay kit is disclosed, which comprises a first antigen bound to a solid phase insoluble in the fluid to be tested and a reagent containing a second antigen bound to a label, wherein the solid phase and the reagent are present in sufficient amounts to perform at least

one assay for analyte antibody in th fluid, and wherein the first and second antigens are the same before they are bound to the solid phase or are labelled, respectively.

In another embodiment, a process is disclosed, whereby the process is for the determination of the presence or concentration of an analyte antibody in a fluid comprising the following steps: a) simultaneously contacting a sample of the fluid with a first and second antigen, wherein the first antigen is bound to a solid phase insoluble in the fluid and the second antigen is labelled and provided in a measured in order to form a insoluble complex between the first and second antigens and the analyte antibody; b) separating the solid carrier from the fluid sample containing unreacted second, labelled antigen; c) measuring the amount of the second, labelled antigen associated with the solid phase or the amount of unreacted second, labelled antigen; d) relating the amount of second labelled antigen with the amount of labelled antigen measured for a control sample prepared in accordance with steps (a) through (c), the control sample known to be free of analyte antibody, to determine the presence of analyte antibody in the fluid sample, or relating the amount of labelled antigen measured for the fluid sample with the amount of labelled antigen measured for samples containing known amount of analyte antibody prepared in accordance with steps a) through c) to determine the concentration of the analyte antibody in the fluid sample; wherein the first antigen and second antigen are the same before they are bound to the solid phase or labelled, respectively.

In alternative embodiments, the label is an enzyme or radioisotope; further, the first and second antigen may bind to antibodies that in turn bind to epitopes of *Borrelia burgdorferi*. In another variation, the label is alkaline phosphatase and the solid phase is a plastic bead, the inner walls of a test tube, the wells of a microtitre plate. In yet another embodiment, the label is alkaline phosphatase and the solid phase is a non-chromatographic device.

Another embodiment discloses a process for the determination of the presence or amount of an analyte antibody in a fluid sample comprising the steps of: a) contacting a sample of the fluid with a first antigen, wherein the first antigen is bound to a solid phase insoluble in the fluid, in order to form an insoluble complex between the first antigen and the analyte antibody; b) separating the fluid sample containing the unreacted analyte antibody from the insoluble complex of the analyte antibody and first antigen; c) reacting a measured amount of a second, labelled antigen with the insoluble complex of the first antigen and the analyte antibody to form an insoluble complex composed of the first and second antigens and the analyte antibody; d) separating the solid phase from the unreacted second, labelled antigen; e) measuring either the amount of second, labelled antigen associated with the solid phase or the amount of unreacted second, labelled antigen;

f) relating the amount of second, labelled antigen measured with the amount of labelled antigen measured for a control sample prepared in accordance with steps a) through e) being known to be free of the analyte antibody to determine the presence of analyte antibody, or relating the amount of labelled antigen measured in the fluid sample with the amount of labelled antigen measured for samples containing known amounts of analyte antibody prepared in accordance with steps a) through e) to determine the concentration of analyte antibody in the fluid sample; wherein the first and second antigens, before they are bound to the solid phase or labelled, respectively, are the same.

In various alternative embodiments, the label is an enzyme, a radioisotope, or alkaline phosphatase. In another variation, the first and second antigens are both antigens that complex with epitopes of the *Borrelia burgdorferi* microorganism. Yet another embodiment discloses a process